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# A new genus of neobatrachian frog from southern Patagonian forests, Argentina and Chile

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## Abstract

In 1975 Lynch named a new species of frog based on two specimens from Puerto Eden, Wellington Island, southern Chile, tentatively allocated to the genus *Telmatobius*. *Telmatobius grandisonae* Lynch was later included by the same author in his genus *Atelognathus*. Based on a reappraisal of the type material and the description of the internal and external morphology, karyotype, tadpole morphology and molecular evidence from recently discovered specimens collected at Lago del Desierto, southern Argentina, we describe the monotypic genus *Chaltenobatrachus,* with *Telmatobius grandisonae* (Lynch) serving as the type species. *Chaltenobatrachus* differs from *Atelognathus* mainly in having a uniform bright green dorsal coloration, with brown to reddish warts; orange iris with gold spots; fingers with interdigital membrane; frontoparietals well developed; small nasals;well ossified sphenethmoid; anteriorly expanded homosternum; skin of tadpole transparent; oral disc with protruding anterior and lateral papillae; diploid number 2n = 32 chromosomes. The genetic distances between *Chaltenobatrachus* and *Atelognathus* meet or exceed most other intergeneric comparisons.

**Key words:** *Chaltenobatrachus* **gen. nov.**, *Chaltenobatrachus grandisonae* **comb. nov.**, Batrachylinae, systematics

## Introduction

Lynch (1975) described the species *Telmatobius grandisonae* based on two preserved specimens collected during December 1958 at Puerto Eden, Wellington Island, by the Royal Society Expedition to South Chile. Both specimens, an adult male (holotype) and a juvenile female (paratype), are deposited at the British Museum (Natural History).

The same author (Lynch 1978), in a re-evaluation of the relationships and classification of the telmatobiine leptodactylid frogs from Patagonia, placed *grandisonae* in his new genus *Atelognathus*, but he remarked that *Atelognathus grandisonae*, the most southern species of the genus, differs from all other species of *Atelognathus* mainly in some osteological characters, and stated that "more material is required to verify the generic assignment".

In January 1997, during field work in the area of Lago del Desierto (49º04'41''S, 72º54'17''W), Santa Cruz Province, southern Patagonia, Argentina (Figure 1), one of us (CAU) collected several adults and tadpoles of a neobatrachian frog with a combination of traits that we could not assign to any of the described genera. The external characteristics of the adult specimens resemble Lynch´s description of the species *A. grandisonae*. In 1997, during a stay at the Field Museum of Natural History, one of us (NGB) had the opportunity to receive on loan the holotype and paratype of *A. grandisonae* through the generosity of Drs. Barry T. Clarke and Colin McCarthy. In January 2005 and 2007 two new field trips were carried out to obtain more specimens for DNA and karyological studies and a comprehensive knowledge of their distribution and habitat.

The specimens collected at Lago del Desierto (Figure 2), along with another specimen deposited at the Argentinean Museum of Natural History (MACN 36084), collected by G. Gil and A. Serret at Lago Nansen (48º5´S, 72º25´W), Perito Moreno National Park, Santa Cruz, Argentina (Cei & Gil 1996) show the same features as species collected at Wellington Island, a locality distant ~100 km airline W from Lago del Desierto. Data from these specimens and their comparison with closely related forms support the recognition of a new genus. Here we provide a

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description based on the recently collected specimens referred to *Telmatobius grandisonae* Lynch in order to supplement Lynch´s original description. We also provide the description of the tadpole, karyotype, adult osteology, and comments on habitat and natural history.


**FIGURE 1.** Distribution of *Chaltenobatrachus grandisonae* in Chile: (1) Isla Wellington, type locality; and Argentina: (2) Lago del Desierto; (3) Lago Nansen.

In the most recent phylogenetic approaches considering the cladistic relationships of the genus *Atelognathus*, Correa *et al.* (2006), Frost *et al.* (2006) and Grant *et al.* (2006) found *Atelognathus* to be closely related to the genus *Batrachyla*, and the subfamily Batrachylinae (Ceratophryidae) was erected to include both genera (Grant *et al.* 2006, Frost 2010). In order to assess the phylogenetic position of the Lago del Desierto frog we conducted a preliminary molecular phylogenetic analysis of representatives of the closely related genera *Atelognathus* and

*Batrachyla*. *Hylorina sylvatica* was included following Correa *et al.* (2006) and *Pleurodema thaul* was used to root the trees.

**FIGURE 2.** *Chaltenobatrachus grandisonae* from Lago del Desierto locality, female (46 mm SVL), not collected.

## Material and methods

The new collected specimens are deposited at the Herpetological Collection of the Centro Nacional Patagónico (CNP) and at the Museo de La Plata, Argentina (MLP): CNP A–392, 393, 342, 343, MLP A–5259, 5 adult specimens. CNP A–394, 1 juvenile, CNP A–395–400, 6 tadpoles stages 31–39 (Gosner 1960). All specimens were collected from wetlands near Lago del Desierto, Chubut province, Argentina, among coordinates 48º59'42''S to 49º04'41''S and 72º50'20''W to 72º58'24''W. Geographic coordinates were obtained using a Garmin 12XL Global Positioning System (GPS). Measurements were taken with calipers to the nearest 0.1 mm under a stereoscopic microscope. The snout to vent length is abbreviated SVL. Drawings were made with the aid of a camera lucida attached to a Carl Zeiss Stemi SV11 stereoscopic microscope. We cleared and stained one adult specimen (CNP A–392) with alizarin red and alcian blue following Taylor and Van Dyke (1985). The cleared and stained specimen was compared with x–rays plates taken of the type specimens BM 1962.628–1962.629. Tadpole measurements and terminology were based on those of Altig and McDiarmid (1999).

Chromosomes were obtained from 6 specimens (2 males and 4 tadpoles) which were injected intraperitoneally with 0.1% colchicine. After three hours, they were killed by overdose of benzocaine; their intestines were carefully removed. Fragments were hypotonically treated with KCl 0.075M for 30 minutes, then fixed in methanol:acetic acid (3:1) and stored at 4ºC for a few days. Tissues were treated with 60% acetic acid and slides were prepared following Kligerman and Bloom (1977) with modifications, and stained with Giemsa. Centromeric positions were established according to Levan *et al.* (1964), and chromosome nomenclature follows Green and Sessions (1991). Relative lengths were determined according to Bogart (1970). Nucleolar organizer regions (NORs) were stained by the Ag–AS technique of Goodpasture and Bloom (1975). Secondary constrictions were not included in the measurements.

Tissue samples for DNA amplification and sequencing were collected from liver or muscle preserved in ethanol 96º. Whole cellular DNA was extracted using DNEasy Tissue Kit (QIAGEN, Valencia, CA). The entire 12S

ribosomal gene (*12S*), partial regions of the 16S ribosomal gene (*16S*) and cytochrome–b gene (*cytb*) from the mitochondrial genome, and the nuclear protein coding gene rhodopsin (*rhod*) were amplified and sequenced using the following primer sets for a total of 3018 base pairs: *12S*: MVZ59, MVZ50, 12Sa, 12Sb; *16S*: 16SL2a, 16Sbr, 16Sa, 16Sar; *cytb*: MVZ15–L, MVZ16–H (Goebel *et al.* 1999); *rhod*: Rhod1a, Rhod1c (Bossuyt & Milinkovitch 2000). Amplification was carried out in a 25ul–volume reaction using standard PCR conditions (Palumbi 1996) with the following thermal cycle profile: 2min at 94ºC, followed by 35–40 cycles of: 94ºC for 30s, 48–56ºC for 30s, and 72ºC for 60s, and a final extension step at 72ºC for 6min. Amplified products were purified using QIAQuik PCR Purification Kit (QIAGEN) and labeled with ABI Prism Big Dye Terminator v. 1.1 chemistry (Applied Biosystems, Foster City, CA). The products were sequenced in both directions on an ABI 3130 genetic analyzer (Applied Biosystems). Contiguous sequences were made using DNA Baser v. 3 (Heracle BioSoft, Pitesti, Romania) and sequences were aligned with Clustal X (Thompson *et al.* 1997). The phylogenetic analysis was performed under maximum-parsimony using TNT, v. 1.1 (Goloboff *et al.* 2003), employing the implicit enumeration option, wich ensures that all minimal length trees are found. We calculated trees with gaps treated as a fifth character. Support estimation was done for a total of 1000 replicates of Parsimony Jackknife, with 0.36 of removal probability. We also performed Bayesian analyses under the model chosen with jModelTest v. 0.1.1 (Posada 2008). Bayesian analyses were performed in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) for each gene an for the combined matrix. Analyses consisted of four heated Markov chains (using default heating values) run for 10 million generations, with Markov chains sampled at intervals of 1000 generations (with a burnin fraction of 0.25).

## Results

## Systematics

### *Chaltenobatrachus* gen. nov.

**Type species.** *Telmatobius grandisonae* Lynch, 1975
**Content.** The genus is monotypic

**Definition and diagnosis.** Size small to medium, up to 46 mm SVL. Dorsal coloration uniform bright green, with brown to reddish warts. Dorsal skin thin and mucoid, finely granulated. Iris orange. Vomerine teeth in two patches between and at posterior level of the choana. Tympanum and columella absent. Fingers with evident interdigital membrane. Plantar skin turgid with low metatarsal tubercles. Supernumerary palmar and plantar tubercles absent. Length of toes in increasing order: 1–2–5–3–4. Frontoparietals moderately extensive, exposing a thin frontoparietal fontanelle. Nasals relatively small, separated medially. Independent quadratojugal absent. Alary processes of premaxillae extensive, directed dorsally. Maxillary teeth extending up to the middle of the orbit. Palatines relatively long, reaching the maxilla, bearing well developed anterior processes. Sphenethmoid well ossified, extending anteriorly beyond the anterior edge of nasals. Cotylar facets of atlas narrowly separated. Transverse processes of posterior presacral vertebrae slightly shortened. Omosternum cartilaginous, elongate, with anterior end expanded. Inner metacarpal with a distinct distal flange at the medial margin of the bone. Terminal phalanges knobbed, not T–shaped. Tadpole with ventral and lateral skin transparent. Anterior and lateral papillae of oral disc protruding from the contour of the snout in dorsal view. Chromosome number 2n = 32.

**Etymology.** The genus name derives from Chaltén, the name given by Tehuelche natives to the main mountain located south of Lago del Desierto, also known as Mount Fitz Roy, of 3406 m elevation.

### *Chaltenobatrachus grandisonae* (Lynch 1975) comb. nov.

*Telmatobius grandisonae* Lynch (1975)
*Atelognathus grandisonae* Lynch (1982)
*Alsodes monticola* Cei and Gil (1996)

**New specimens.** CNP A–392, 393, 342, 343, 394–400 collected 27 January 1997, Lago del Desierto, Santa Cruz Province, Argentina (49º04’41’’S, 72º54’17’’W).

**Description** (based on types and new available specimens). Small to medium frog (adults up to 46 mm SVL; Table 1) with frog–like appearance, body and limbs of regular proportions. Head somewhat smaller than one third of body length, depressed, rounded in dorsal view, wider than long. Snout shorter than ocular diameter, rounded to slightly truncate in dorsal view; sloping, with a noticeable angle at the level of the nostrils in lateral profile. Upper lip slightly protruding over lower lip. Nostrils oval, somewhat protuberant, directed dorsolaterally, approximately equidistant between the tip of the snout and the eye. Canthus rostralis rounded, loreal region weakly concave and strongly sloping towards the lip. Eyes large, very prominent and fairly lateral (in dorsal view protruding from the mandibular branches), oriented laterally. Pupil rhomboidal; a small and round ventral pupillary nodule on lower border of iris evident in some specimens. Interocular distance smaller than upper eyelid width, and similar to internarial distance. Tympanum absent. Supratympanic fold noticeable and thick, extending from the posterior end of the eye to the insertion of the arm. Tongue rounded, somewhat emarginate posteriorly (notched in exemplar MLP A–5259), free laterally and posteriorly. Premaxillary and maxillary teeth large; vomerine teeth in two oval, prominent patches, located close together, between and at posterior level of the choana. Choanae rounded, not concealed by the pars palatine of maxilla. Arms thin and of moderate length. Fingers moderately long, with noticeable interdigital membrane, more developed between the pollex and second finger and between the second and third fingers; tips of fingers rounded (Figure 3a). Pollex very wide at its base. Length of fingers in increasing order: 1=2–4–3. Metacarpal tubercles large but low, the inner oval, the external weakly bifid; subarticular tubercles large and rounded or wider than long, the proximal ones larger and more protuberant; supernumerary palmar tubercles absent. Forearms somewhat more robust than arms. Legs thin and of moderate length. Toes depressed, with thick interdigital membrane, deeply incised, continuing as a dermal fringe on all toes until almost the rounded tip. Toe V with narrow dermal fringe. Length of toes in increasing order: 1–2–5–3–4. Plantar skin turgid; two little, low, noticeable metatarsal tubercles (Figure 3a), the inner one oval and the outer one smaller and circular; subarticular tubercles small, rounded; supernumerary plantar tubercles absent. Tarsal fold noticeable, extending along most of the tarsus and continuous with the dermal fringe of the first toe. Tibiotarsal articulation reaching the eye. When femurs and tibias placed at right angle to the sagittal plane, the tibiotarsal articulations not in contact. Skin thin and mucoid. Back of the head, dorsum, and legs finely granulated with numerous warts, soft and turgid, of variable size and shape (rounded or elongated). Ventral skin smooth and loose; seat patch with turgid vascularized warts, restricted to the lower side of the thighs near the vent. Gular fold faint. Males with nuptial excrescences in the form of asperities, little pigmented, wide on the dorsal and inner side of the pollex and less extensive on the inner side of the second finger; also reaching the inner edge of the inner metacarpal tubercle.

**Color in life.** Back of the body and limbs fairly uniform bright green color, with brown to reddish warts (Figure 2). Dark, diffuse transverse bands on the dorsal side of the legs, more evident in juveniles. Belly and lower part of the flanks greyish, evenly speckled with tiny black spots. Throat whitish speckled with small dark spots. Ventral skin of the legs brownish and translucent. A diffuse dark brown band from the tip of the snout to the eye, including the nostrils, continuing to the axillary region along the supratympanic fold. A dark patch under the eye reaching the upper lip. Iris orange with gold spots; a dark vertical line under the pupil. Metamorphs show the typical species coloration. Juveniles with more intense colouring of the transverse bands of the legs, the dorsal warts and the bands on the head.

**Osteology**. (Description based on CNP A–392, adult male). Skull as long as wide (Figure 3b). Frontoparietals paired, moderately extensive, exposing a thin frontoparietal fontanelle (frontoparietals nearly contacting each other in holotype), widely separated from nasals. Nasals relatively small, separated from one another medially and from the pars facialis of the maxilla laterally. Maxillary arch incomplete, lacking independent quadratojugal. Alary processes of premaxillae extensive, directed dorsally. Pars dentalis of premaxilla bearing 9–11 well developed pedicellate teeth; pars palatina narrow, with prominent palatine process. Pars facialis of maxilla rectangular; pars dentalis bearing 26–28 teeth extending up to the middle of the orbit; pars palatina narrow, no pterygoid processes developed. Parasphenoid triradiate; cultriform process lanceolate, not reaching anteriorly the level of the planum antorbitale; parasphenoid alae directed laterally. Vomers relatively small, widely separated medially; dentigerous processes situated between and partially posterior to choanae, each bearing 2–3 well developed teeth; prechoanal, postchoanal, and anterior processes of vomer well developed; anterior processes widely separated from the maxillary arcade. Palatines relatively long, reaching the maxilla, bearing well developed anterior processes. Pterygoids triradiate; anterior ramus of pterygoid in contact with maxilla, not reaching the planum antorbitale; medial ramus of pterygoid in contact with otic capsule but not bearing a bony articulation. Zygomatic rami of squamosals relatively

long; otic rami shorter than zygomatic rami, overlapping the cartilaginous cristae parotica. Sphenethmoid well ossified, extending anteriorly notably beyond the anterior edge of nasals, with an anteromedial ossification between the nasal capsules; bony sphenethmoid not reaching the anterior margin of optic foramen posteriorly. Prootics fused with exoccipitals; epiotic eminences well developed. Occipital condyles lacking a constricted base and slightly separated medially. Opercula present, not mineralized. Columellae and tympanic annuli absent.

Hyoid plate width approximately equal to medial length (Figure 3c); hyalia long, lacking anterior processes; anterolateral and posterolateral hyoid processes present, long and slightly expanded distally; posteromedial hyoid processes long and well ossified.

Eight procoelous, nonimbricate presacral vertebrae. Cotylar facets of atlas narrowly separated, approaching a Type II condition (Lynch 1971). Transverse processes of presacrals II–IV broader than those of vertebrae V–VIII. Transverse processes of presacrals II, VII and VIII distinctly oriented anteriorly; processes of presacrals V–VIII slightly shortened. Sacral diapophyses slightly deflected posteriorly; moderately dilated. Sacrococcygeal articulation bicondylar. Urostyle with small dorsal crest on proximal half.

Pectoral girdle arciferal (Figure 3d). Omosternum cartilaginous; elongate, with anterior end expanded. Scapula bicapitate. Clavicle arched, not overlaying the pars acromialis of the scapula. Coracoid narrowly dilated at its distal and proximal ends. Sternum cartilaginous, expanded posterolaterally. All cartilage of pectoral girdle calcified to some degree in adult specimen examined. Cleithrum cleft distally.

Ilial shaft cylindrical, elongate; dorsal crest poorly developed; dorsal prominence directed dorsolaterally; protuberance evident; preacetabular angle approximately 90°. Ischia small, lacking prominent processes. Pubis cartilaginous, calcified.

Humerus slender, bearing distinct crests; crista ventralis moderate; crista medialis slightly larger than crista lateralis. Phalangeal formula of hand 2–2–3–3. Terminal phalanges knobbed. Prepollex with two ossified elements. Inner metacarpal with a notorious distal flange at medial margin of the bone. Prehallux with one basal element ossified and three distal cartilaginous elements. Phalangeal formula of foot 2–2–3–4–3.

**Tadpole** (based on 6 specimens at stages 31 to 39 sensu Gosner (1960), CNP A–395–400) Medium–sized larva (up to 56 mm) (Figure 4a,b; Table 2). Body elliptic in dorsal view, with a slight constriction posterior to the head; body 2 times longer than high, somewhat depressed (1.2 times wider than high), 35–40% of total length. Snout rounded in dorsal and lateral view. Nostrils small and circular, with an elevated internal rim, in dorsolateral position, slightly closer to the snout than to the eyes, opening into a depression, not raised. Distance between nostrils smaller than distance between eyes (0.4 to 0.6 times). Eyes large, in lateral position, oriented dorsolaterally, small umbraculum (fleshy projection of the iris over part of the pupil) present. Spiracular tube sinistral and conspicuous; the distal end free from the body wall. Spiracle posterolaterally directed, located beneath the mid-line of the body, 46–62% of body length, inner wall free. Vent tube dextral, short, with dextral opening.

Tail slightly higher than the body, about 60–65% of total length. Caudal musculature robust, not reaching the tip of the tail. Tail axis straight. Dorsal and ventral fins well developed, of similar height, with gently arched margins; dorsal fin arising from the last part of the body or at the tail-body junction; ventral fin arising at the level of the cloacal opening. Tip of the caudal fin rounded.

Oral disc subterminal, ventral (Figure 4c), emarginated (with lateral constrictions). Single row of marginal papillae, with very large rostral gap (62–78% of the oral disc width). In dorsal view anterior and lateral papillae protruding from the contour of the snout. Supra-angular, angular and infra-angular intramarginal lateral papillae present, the latter conspicuous and numerous. All papillae sharp-pointed. Intramarginal mental papillae absent. Labial teeth well developed and keratinized. Labial tooth row-formula 2(2)/3(1). P1 gap very narrow. Upper and lower jaw sheaths well developed, wider than high, with finely serrate margins; lower jaw sheath strongly curved. Individuals raised through metamorphosis in the laboratory had the morphological and coloring features that allowed them to be identified as the same species. Metamorphs (stage 46) measure between 14.9 and 16.3 mm.

**Color of tadpole.** In life, dorsal color dark brown with golden spots (Figure 4a). Ventral and lateral skin transparent, forming a distinct translucent contour around the body. Ventral surface of the head unpigmented, with chondrocranium, gills and heart visible through transparent skin. Abdomen black with few groups of golden, superficial guanophores. Caudal musculature dark brown with irregular golden patches. Fins translucent with scattered melanophores along the course of blood vessels, and scattered groups of guanophores. Iris gold, with dark spots. Nostrils with a pigmented rim. Spiracular tube transparent. Keratinized buccal structures highly pigmented.

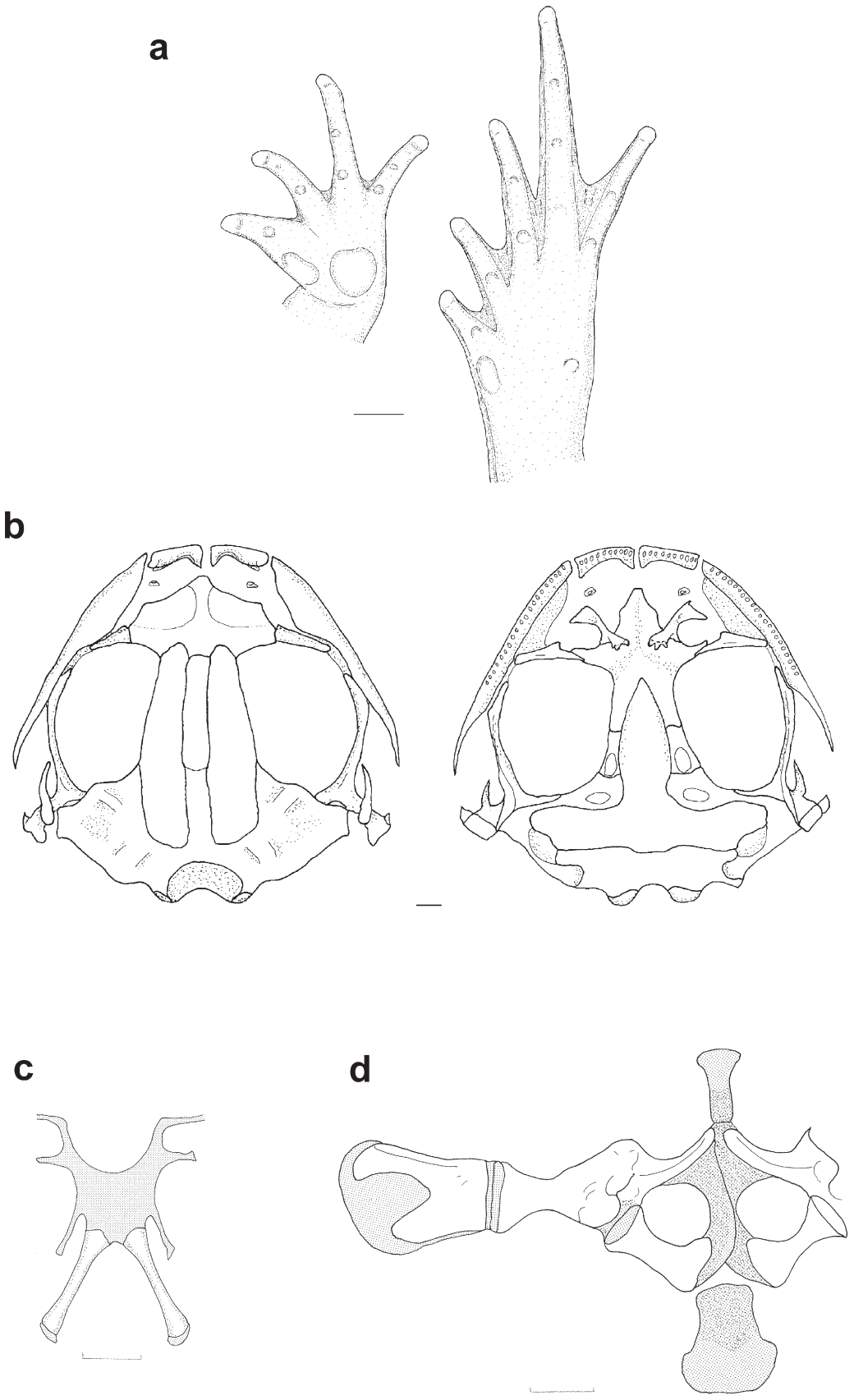


**FIGURE 3.** (a) Ventral view of hand (left) and foot (right) of *Chaltenobatrachus grandisonae*, MLP A–5259. (b) Dorsal (right) and ventral (left) views of skull of *Chaltenobatrachus grandisonae*, CNP A–392. (c) Hyoid of *Chaltenobatrachus grandisonae*, CNP A–392. (d) Ventral view of pectoral girdle and sternum of *Chaltenobatrachus grandisonae*, CNP A–392. Scale = 2 mm.

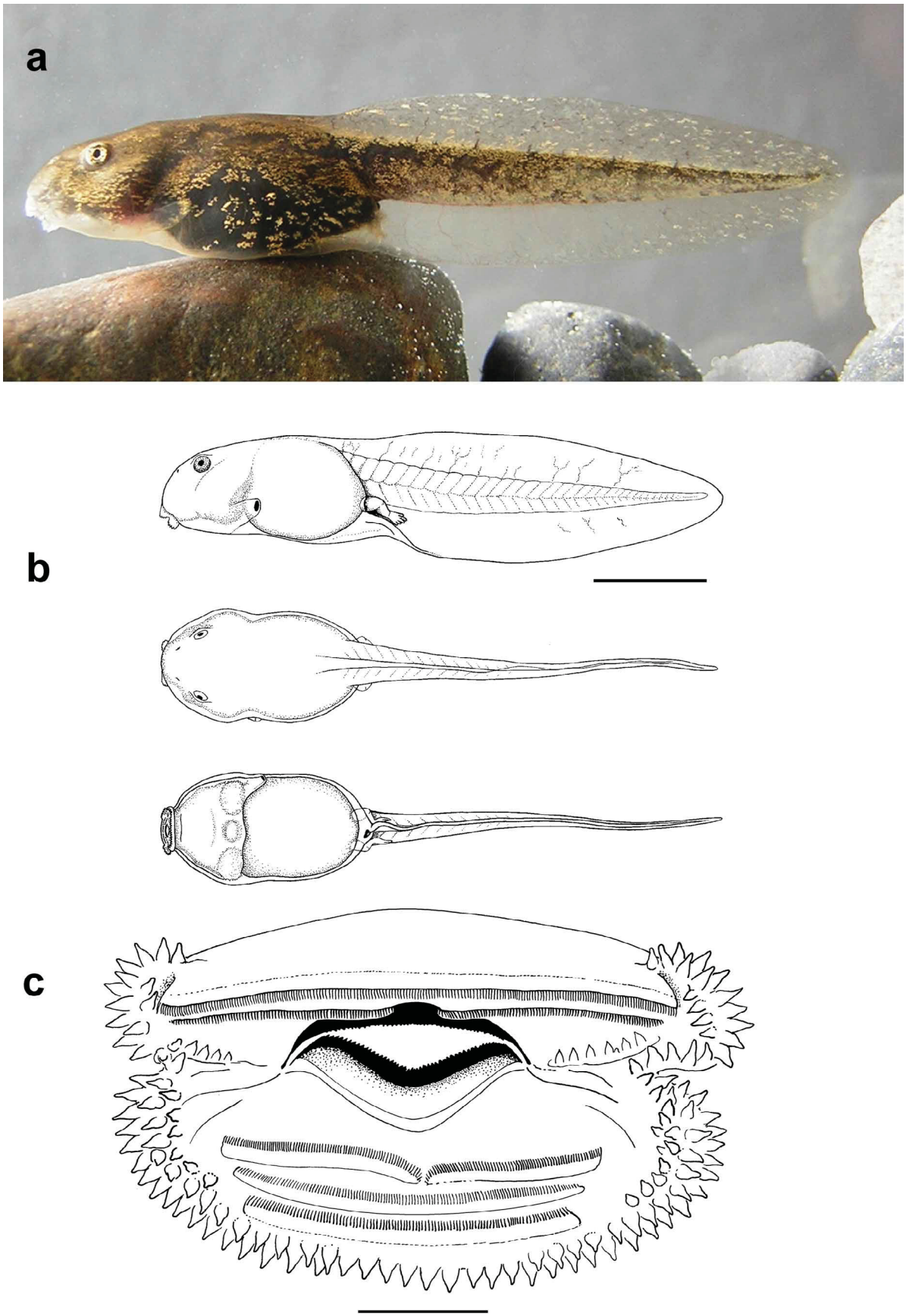


**FIGURE 4.** (a) *Chaltenobatrachus grandisonae* larva, stage 26, 37 mm total length, not collected. (b) *Chaltenobatrachus grandisonae* larva, CNP A–397, stage 38. Scale = 10 mm; lateral view (upper); dorsal view (middle); ventral view (lower). (c) Oral disc of *Chaltenobatrachus grandisonae* larva, CNP A–398, stage 39. Scale = 1 mm.

**Karyology.** *Chaltenobatrachus grandisonae* has a diploid complement of 32 chromosomes in 87 metaphases. The analysis of 16 metaphasic plates revealed a fundamental number (FN) of 60. Pairs 7, 9, 10, 11, 12, 14 and 15 are metacentric (m); pairs 1, 6 and 8 are submetacentric (sm); pairs 2, 3, 4 and 5 are subtelocentric (st) and pairs 13 and 16 are telocentric (t) (Figure 5a; Table 3). Pair 7 has a secondary constriction (SC) on the large arm in interstitial position in all plates studied. This SC shows an achromatic gap variable in size in the homologous pair. The specimens showed active ribosomal cistrons on the secondary constriction (Figure 5b). When chromosomes are arranged according to their decreasing length, pairs 1 and 2 are large (>100 units), pair 3 is intermediate (between 80 and 100 units) and pairs 4 to 16 are small (<80 units) (Table 3). Thirty six percent of the length of the karyotype is taken up by the three first large-intermediate pairs of chromosomes, while sixty four percent is taken up by the thirteen small chromosomes.

**TABLE 1.** Measurements of *Chaltenobatrachus grandisonae* adult specimens. SVL: snout-vent Length; HL: head length; HW: head width; ED: eye diameter; END: eye-naris distance; UEL: upper eyelid length; UEW: upper eyelid width; IOD: interorbital distance; IND: internarial distance; SED: snout-eye distance; TL: tibia length; FoL: foot length; FL: femur length. * Cleared and stained.

| Character | BM 1962.629 | CNP 209 | A– | CNP 210 * | A– | CNP 211 | A– | CNP 212 | A– | MLP 5259 | A– | MACN 36084 | Average ± SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SVL | 33.3 | 38.2 | | 38.5 | | 37.5 | | 40.3 | | 34.6 | | 37.5 | 37.13 ± 2.39 |
| HL | 11.7 | 11.4 | | 11.8 | | 10.8 | | 11.4 | | 11.7 | | 10.1 | 11.27 ± 0.62 |
| HW | 11.7 | 11.8 | | 12.3 | | 10.9 | | 11.2 | | 11.2 | | 10.3 | 11.34 ± 0.66 |
| ED | 3.7 | 3.6 | | 3.2 | | 3.5 | | 3.5 | | 3.6 | | 3.5 | 3.51 ± 0.16 |
| END | 2.5 | 2.7 | | 2.4 | | 2.5 | | 2.4 | | 2.9 | | 2.3 | 2.53 ± 0.21 |
| UEL | 4.9 | 5.8 | | 5.7 | | 5.3 | | 5.1 | | 4.8 | | 4.8 | 5.20 ± 0.42 |
| UEW | 3.6 | 2.6 | | 3.3 | | 2.5 | | 3.2 | | 3.4 | | 2.4 | 3.00 ± 0.49 |
| IOD | 3.3 | 3.1 | | 3.1 | | 3.2 | | 3.1 | | 2.4 | | 2.7 | 2.99 ± 0.32 |
| IND | 2.7 | 2.5 | | 2.5 | | 2.4 | | 2.6 | | 2.5 | | 2.1 | 2.47 ± 0.19 |
| SED | 5.1 | 4.9 | | 5.1 | | 4.8 | | 5.1 | | 4.6 | | 4.5 | 4.87 ± 0.25 |
| TL | 14.6 | 15.2 | | 15.2 | | 15.2 | | 15.8 | | 15.7 | | 14.4 | 15.16 ± 0.52 |
| FoL | 21.8 | 24.3 | | 23.7 | | 25.1 | | 24.3 | | 24.4 | | 22.2 | 23.69 ± 1.23 |
| FL | 15.2 | 16.8 | | 17.2 | | 17.4 | | 14.1 | | 17.4 | | 14.5 | 16.09 ± 1.44 |

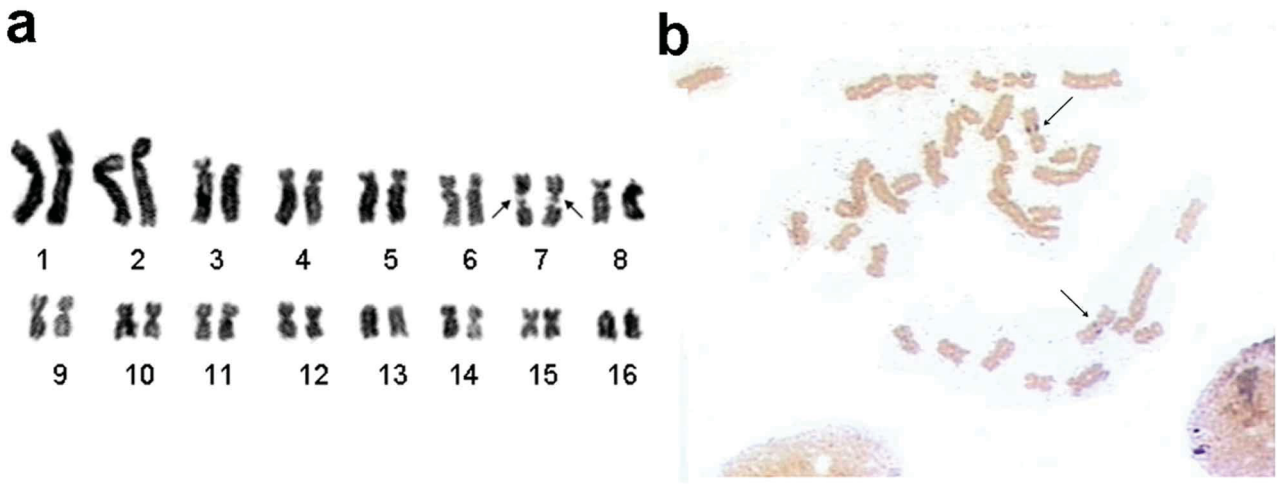


**FIGURE 5**. (a) Karyotype of *Chaltenobatrachus grandisonae.* Arrows show secondary constrictions. (b) Ag-NOR stained metaphase of *Chaltenobatrachus grandisonae.* Arrows show active ribosomal cistrons.

**TABLE 2**. Average ± standard deviation of the morphometric variables of *Chaltenobatrachus grandisonae* larvae. Stages were assigned according to Gosner (1960). Measurements are expressed in mm.

| Measurements | Stage | | | |
|---|---|---|---|---|
| | 31 (n=1) | 37 (n=1) | 38 (n=1) | 39 (n=3) |
| Total length | 39.5 | 49.8 | 50.2 | 49.25±1.72 |
| Body length | 16.2 | 19.5 | 18.9 | 17.94±0.56 |
| Tail length | 23.3 | 30.3 | 31.3 | 31.31±1.65 |
| Maximum body height | 8.8 | 10.3 | 8.4 | 8.91±0.27 |
| Maximum tail height | 7.8 | 10.5 | 10.3 | 9.2±0.55 |
| Caudal musculature height | 2.8 | 3.4 | 3.16 | 3.42±0.09 |
| Maximum body width | 10.2 | 11.6 | 11.0 | 11.04±0.3 |
| Eye diameter | 1.3 | 1.7 | 1.6 | 1.85±0.15 |
| Interorbital distance | 3.4 | 3.8 | 3.5 | 4±0.3 |
| Extraocular distance | 6.3 | 7 | 6.8 | 7±0.1 |
| Body width (eye level) | 8.2 | 9.9 | 9.51 | 9.57±0.26 |
| Nostril diameter | 0.3 | 0.2 | 03 | 0.26±0.04 |
| Internarial distance | 2.1 | 2 | 2 | 2.05±0.13 |
| Extranarial distance | 2.5 | 2.7 | 2.5 | 2.4±0.41 |
| Body width (nostril level) | 5.5 | 6.5 | 5.6 | 6.9±0.66 |
| Rostronasal distance | 1.7 | 1.7 | 1.6 | 1.65±0.24 |
| Orbitonasal distance | 1.5 | 2.3 | 1.9 | 2.25±0.13 |
| Rostroorbital distance | 3.7 | 4 | 4 | 4±0.47 |
| Rostro-spiracular distance | 8.3 | 9.0 | 10.5 | 10.55±0.66 |
| Oral disc width | 4.2 | 4.4 | 4.6 | 4.5±0.05 |
| Rostral gap width | 2.7 | 3.3 | 3.2 | 2.9±0.33 |
| Suprarostrodont length | 1.9 | 2.2 | 2 | 1.95±0.1 |
| Infrarostrodont length | 1.5 | 2 | 1.5 | 1.7±0.05 |

**Phylogenetic relationships.** The DNA dataset included a total of 3018 base pairs (933bp from *12S*, 1036 from *16S*, 707 from *cytb*, and 342 from *rhod*), of which 384 sites were informative under parsimony. The Maximum Parsimony analysis by TNT resulted in a single most parsimonious tree of 1134 steps (ci= 0.786, ri= 0.688). In our cladogram *Chaltenobatrachus grandisonae* is the sister group of the genus *Atelognathus*. *Hylorina sylvatica* is sister to *Batrachyla*, and this clade forms a strongly supported group with the clade formed by *Chaltenobatrachus* + *Atelognathus* (Figure 6). All nodes are strongly supported. The genus *Atelognathus* shows a MP Jackknife value of 100 %, corroborating its monophyletic nature. For Bayesian inference analyses, the best model for our data was GTR + G. The Bayesian analyses recovery the same topology as was estimated using Maximun Parsimony in TNT, for the combined matrix as for each gene partition. The only exception is the *cytb* partition, where *Chaltenobatrachus* forms a polytomy with the monophyletic genus *Atelognathus* and the clade containing *Hylorina* and *Batrachyla.* All nodes in the combined matrix resulted in posterior probability values of 100%. We adopt the subfamily name Batrachylinae for the clade containing *Batrachyla*, *Hylorina*, *Chaltenobatrachus* and *Atelognathus* (see Figure 6).

**Habitat and natural history.** *Chaltenobatrachus grandisonae* lives in rain forests and wetlands of the southern Andes Mountains in Patagonia, at least from 48° to 49°S. Regarding altitude, it has been found from the level of Lago del Desierto (500 m a.s.l.) to very near the treeline (830 m a.s.l.). The type locality –plateau below the south peak (640 m a.s.l.), Puerto Edén on Isla Wellington– was described by Grandison (1961), extracted from a personal communication from Dr. Holdgate, zoologist with the Royal Society Expedition to Southern Chile.

The type locality and the new localities described here, Lago del Desierto and Lago Nansen, are located in a mountainous area showing strong evidence of glaciation, near large ice fields. Permanent snow and glaciers are

present at the summits of many of the higher elevations. The climate in the area is cold and wet, with long winters and precipitation in the form of abundant snow.

Valleys and slopes up to about 1000 m elevation are covered by temperate-cold rain forests of austral beeches (*Nothofagus* spp.). At the type locality there are tangled dwarf thickets of *Nothofagus antarctica*, alternating with open moorlands (Grandison 1961). At the localities Lago del Desierto (Figure 7) and Lago Nansen (Cei & Gil 1996) there are deciduous forests of *Nothofagus pumilio* with a sparse shrub stratum and a herbaceous stratum rich in pteridophytes and bryophytes, which can form dense mats covering fallen logs and standing tree trunks. In poorly drained areas around wetlands, *Nothofagus antarctica* is present. There are many bodies of water such as lakes of glacial origin, ponds and streams. On flat sites or those with poor drainage, communities are predominantly herbaceous hygrophytic, made up of grasses, rushes and sedges (Gramineae, Juncaceae and Cyperaceae), and peat bogs. Smaller bodies of water freeze superficially in winter.

**TABLE 3**. Centromeric Index, relative length (mean and standard deviation), and types of chromosomes of *Chaltenobatrus grandisonae* **sp. nov.** [a] Centromeric Index = short arm/total length of the chromosome. [b] Relative length according to Bogart (1970). [c] m : metacentric, sm : submetacentric, st : subtelocentric, t : telocentric. * Chromosomes with secondary constriction.

| Chromosomes | Centromeric Index [a] | Relative length [b] | Type[c] | Type of chromosome |
|---|---|---|---|---|
| 1 | 31.9 ± 4.0 | 140.8 ± 1.8 | sm | large |
| 2 | 24.7 ± 1.8 | 132.3 ± 1.2 | st | large |
| 3 | 20.0 ± 3.4 | 85.4 ± 1.0 | st | Intermediate |
| 4 | 23.6 ± 4.2 | 73.4 ± 0.5 | st | small |
| 5 | 22.5 ± 4.7 | 66.6 ± 0.4 | st | small |
| 6 | 26.7 ± 4.6 | 61.1 ± 0.6 | sm | small |
| 7 * | 39.2 ± 4.2 | 55.4 ± 0.5 | m | small |
| 8 | 28.5 ± 5.2 | 51.7 ± 0.6 | sm | small |
| 9 | 41.8 ± 3.7 | 48.6 ± 0.4 | m | small |
| 10 | 41.7 ± 3.2 | 43.6 ± 0.3 | m | small |
| 11 | 39.0 ± 4.2 | 41.5 ± 0.3 | m | small |
| 12 | 42.7 ± 3.8 | 39.8 ± 0.3 | m | small |
| 13 | | 39.0 ± 0.6 | t | small |
| 14 | 43.4 ± 3.6 | 38.0 ± 0.5 | m | small |
| 15 | 45.1 ± 1.6 | 33.8 ± 0.4 | m | small |
| 16 | | 29.1 ± 0.4 | t | small |

Juveniles and adults of *Chaltenobatrachus grandisonae* were found under fallen logs near small water bodies, and adults were found submerged in pools, probably engaging in breeding activity. During three different summers (January) tadpoles were found in a variety of lentic environments located between 500 and 800 m a.s.l., either in wetlands or in open areas in the forest (Figure 7a). These include small, shallow pools (3–10 m diameter, 10–20 cm depth pH = 6.5) and larger pools (20 m diameter, 1 m depth, pH = 6.5) with submerged macrophytes (*Miriophyllum*) and spherical *Nostoc* sp. colonies. Tadpoles were also found in the forest in small, cold, shady pools (2 m diameter, 30 cm depth, pH = 6.2), surrounded by liverworts (Hepaticae) and *Gunnera magellanica*.

There are no data on the breeding behaviour of *Chaltenobatrachus grandisonae*. Mating call, amplexus type and egg laying are still unknown. Once, the release call of a female was heard. Larval development is aquatic and the larva is an active feeder. In summer (January) different sized tadpoles were found living together: small (18–20 mm, development stage 25), and large (up to 56 mm, stages 37 to 39). The fact that there were larvae from different cohorts present at the same time allows us to assume that they developed from clutches laid in different years, and the larvae have a prolongued development and overwinter in the water bodies. Metamorphosis must occur at the end of summer.

*Chaltenobatrachus grandisonae* was found sympatrically with an *Alsodes* species and *Nannophryne variegata*. Grandison (1961) also cited these two species (*Alsodes* sp. cited as *Eupsophus coppingeri*) for the area of the type locality on Wellington Island. In the area of Lago del Desierto, *Chaltenobatrachus grandisonae* tadpoles were found cohabitating the shallowest pools with *Nannophryne variegata* tadpoles.

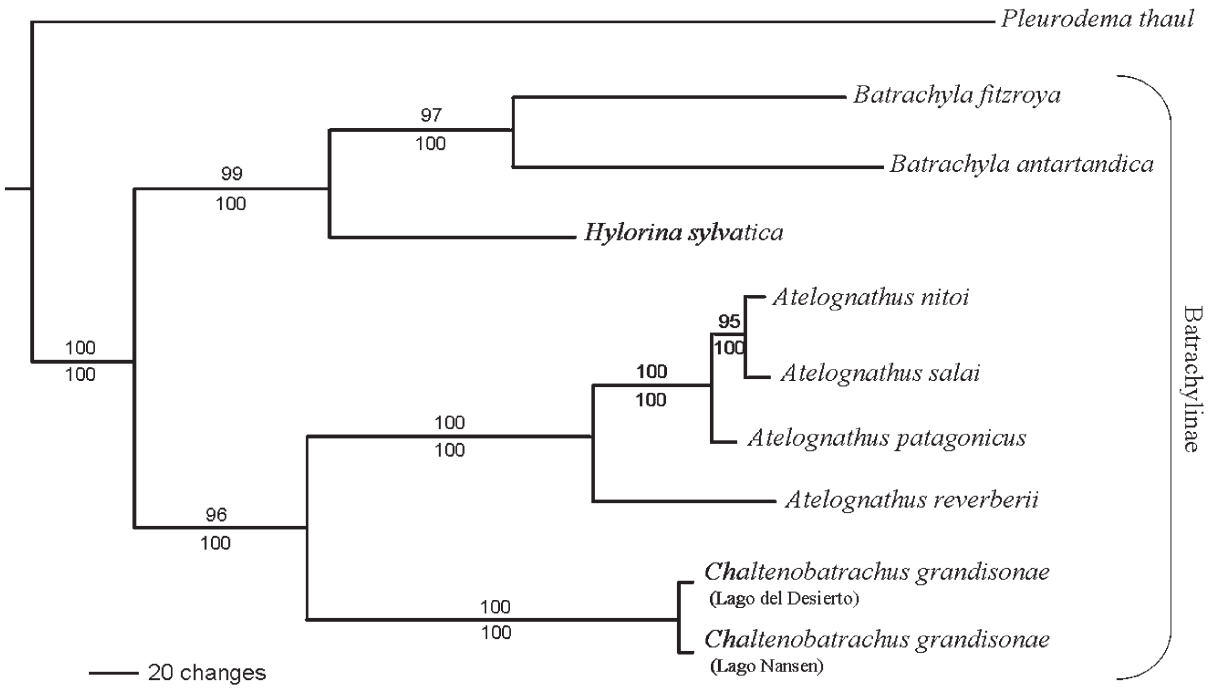


**FIGURE 6.** Maximum parsimony tree of combined molecular data depicting the relationship of *Chaltenobatrachus grandisonae* within Batrachylinae. Numbers above branches indicate Jackknife support values; numbers below branches indicate Bayesian posterior probabilities.

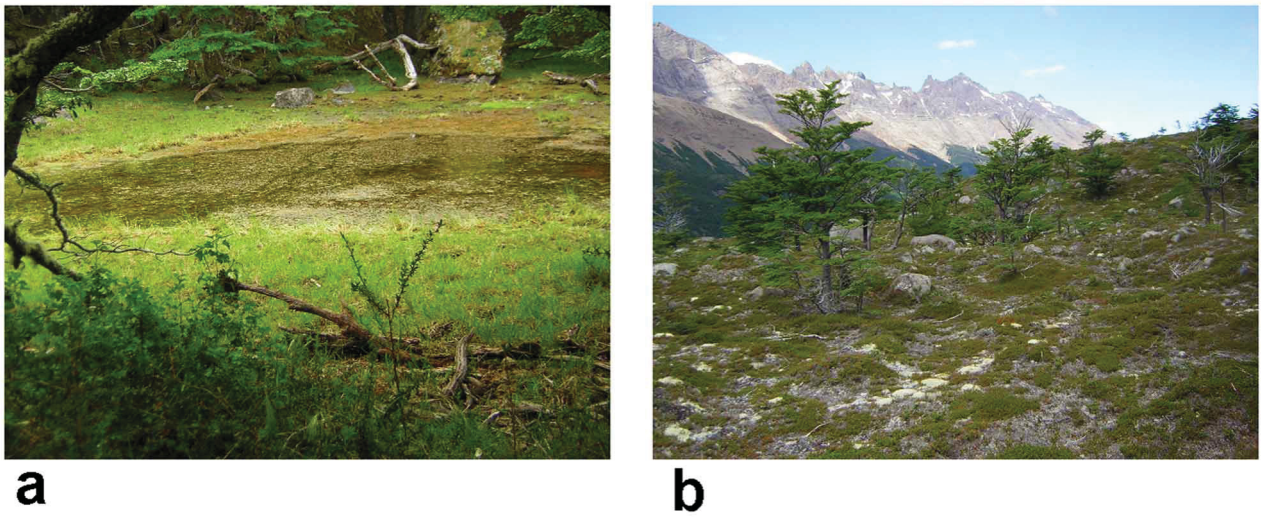


**FIGURE 7.** (a) Temporary pond in an open area of the austral beech, *Nothofagus pumilio*, forest near Lago del Desierto, Chubut, Argentina. Reproductive and developmental habitat of *Chaltenobatrachus grandisonae*. (b) General view of a rainforest of the austral beech, *Nothofagus pumilio*.

## Discussion

*Chaltenobatrachus grandisonae* is sister to the clade nesting the species of the genus *Atelognathus*. When Lynch first described *Telmatobius grandisonae* (Lynch 1975) he examined two specimens revised by Alice Grandison,

who thought the frogs might represent an unnamed genus. In a later paper, Lynch (1978) included *grandisonae* within his genus *Atelognathus*, but remarked on several morphological characters that cast doubt on their generic placement (Lynch 1978: 16).

Because of the phylogenetic position of *Chaltenobatrachus grandisonae* as sister to *Atelognathus*, it would be cladistically possible to simply expand the definition of *Atelognathus* to include *Chaltenobatrachus*. Nevertheless, this practice would incorporate a high level of morphological, karyological and ecological variability to the otherwise clearly defined genus *Atelognathus*. The genus *Chaltenobatrachus* is justified because the recognized derived features defining *Atelognathus* (large, exposed frontoparietal fontanelle, short palatine bones and large nasals [Lynch 1978; Basso 1998]) are not present in *Chaltenobatrachus*. *Chaltenobatrachus* differs from *Atelognathus* mainly in having more extensive frontoparietals, small nasals, long palatines, extensive alary processes of premaxillae, maxillary teeth extending up to the middle of the orbit, well ossified sphenethmoid, omosternum anteriorly dilated, fingers webbed, uniform bright green coloration of dorsal skin with brown to reddish warts, orange color of iris, tadpole with transparent skin and protruding lateral papillae of oral disc in dorsal view. The cytochrome-b genetic distances (p-distance) between *Chaltenobatrachus grandisonae* and the species of *Atelognathus* ranges from 17.1% to 17.9%, while the genetic distances among *Atelognathus* species ranges from 1.1% to 8.4%. Similar values of genetic distance at generic level are obtained when comparing *Chaltenobatrachus* vs *Batrachyla* (17.3–19.0%) and *Chaltenobatrachus* vs *Pleurodema* (17.6%).

The presence of 32 chromosomes is an autapomorphy of *Chaltenobatrachus*. The increment in the chromosome number from the basal condition 2n = 26 (Barrio & Rinaldi de Chieri 1971; Cuevas & Formas 2008) present in the current Ceratophryidae (sensu Frost *et al.* 2006; Grant *et al.* 2006) could be achieved by centric fissions followed by pericentric inversions, as only two pairs of telocentric chromosomes are present. Thirteen chromosome pairs take up 64 % of the haploid genome, so, independent segregation of major blocks of genes is not constrained in *Chaltenobatrachus* and could represent an adaptive innovation in the evolution of the genus.

The IUCN Red List of Theatened Species categorized *C. grandisonae* from Chile (named as *Atelognathus grandisonae*) as Data Deficient (Veloso & Núñez 2008). In Argentina, *Chaltenobatrachus grandisonae* occurs in remote, inaccessible, Patagonian territories, including natural protected areas. Therefore, it could be assumed that its habitat is not threatened. On the other hand, the lack of knowledge of its natural history and population biology does not allow us to accurately assess its conservation status, so we recommend maintaining it as Data Deficient.

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